Javor et al. For example, the mouse LPS model of NASH may be a special case, mouse NASH may be fundamentally a poor model of human NASH, or the NASH in lipodystrophy may be unrepresentative of "typical" NASH due to inadequate adipose tissue storage capacity. To determine which (if any) of these explanations is correct will require a deeper understanding of NASH.

A starting point might be detailed phenotyping and classification of NAFLD using criteria in addition to medical history and histology. Comparison with cancer is illustrative: by exquisitely genotyping and phenotyping cancers, treatments can now be tailored, thus yielding a higher probability of treatment success and less toxicity. A similar analysis of NAFLD may identify subclasses of the disease with unique immune, inflammatory, and metabolic characteristics and may reveal whether leptin plays the same or different roles in each type. Genetic screens have now identified six loci that contribute to variance in NAFLD traits (e.g., Romeo et al., 2008; Speliotes et al., 2011), with one increasing hepatic triglyceride production (Kumari et al., 2012), suggesting that further phenotyping efforts will be fruitful. It seems likely that integrating phenotypic and genetic information with mechanistic studies will help unravel the conundrum that is NASH. In summary, Imajo et al. make a strong case that leptin contributes to NASH in a mouse endotoxin model of NASH. This interesting result will spur examination of the generality of the contribution of leptin in other NASH models and drive more detailed mechanistic studies of NAFLD.

REFERENCES

Chalasani, N., Younossi, Z., Lavine, J.E., Diehl, A.M., Brunt, E.M., Cusi, K., Charlton, M., and Sanyal, A.J. (2012). Hepatology *55*, 2005–2023.

Cohen, J.C., Horton, J.D., and Hobbs, H.H. (2011). Science *332*, 1519–1523.

Farooqi, I.S., Matarese, G., Lord, G.M., Keogh, J.M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S.A., Perna, F., Fontana, S., et al. (2002). J. Clin. Invest. *110*, 1093–1103.

Hebbard, L., and George, J. (2011). Nat. Rev. Gastroenterol. Hepatol. *8*, 35–44.

Imajo, K., Fujita, K., Yoneda, M., Nozaki, Y., Ogawa, Y., Shinohara, Y., Kato, S., Mawatari, H., Shibata, W., Kitani, H., et al. (2012). Cell Metab. *16*, this issue, 44–54.

Javor, E.D., Ghany, M.G., Cochran, E.K., Oral, E.A., DePaoli, A.M., Premkumar, A., Kleiner, D.E., and Gorden, P. (2005). Hepatology *41*, 753–760.

Kumari, M., Schoiswohl, G., Chitraju, C., Paar, M., Cornaciu, I., Rangrez, A.Y., Wongsiriroj, N., Nagy, H.M., Ivanova, P.T., Scott, S.A., et al. (2012). Cell Metab. *15*, 691–702.

Romeo, S., Kozlitina, J., Xing, C., Pertsemlidis, A., Cox, D., Pennacchio, L.A., Boerwinkle, E., Cohen, J.C., and Hobbs, H.H. (2008). Nat. Genet. *40*, 1461–1465.

Speliotes, E.K., Yerges-Armstrong, L.M., Wu, J., Hernaez, R., Kim, L.J., Palmer, C.D., Gudnason, V., Eiriksdottir, G., Garcia, M.E., Launer, L.J., et al; NASH CRN; GIANT Consortium; MAGIC Investigators; GOLD Consortium. (2011). PLoS Genet. *7*, e1001324.

Vernon, G., Baranova, A., and Younossi, Z.M. (2011). Aliment. Pharmacol. Ther. *34*, 274–285.

A Role for Period 2 in Cardioprotection

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How adenosine receptors protect the ischemic heart is not completely understood. Eckle et al. (2012) now show that signaling through adenosine receptor 2b (Adora2b) stabilizes a circadian rhythm protein, period 2 (Per2), resulting in the stabilization of hypoxia-inducible factor-1α (HIF-1α), upregulation of glycolysis, and cardioprotection from ischemia.

Myocardial ischemia results in dramatic metabolic perturbations, due primarily to a mismatch between cardiac muscle oxygen supply and demand. The ensuing deficits in ATP production can lead to cardiac myocyte cell death. As such, considerable efforts have focused on modulating cardiac energy metabolism as an approach to treat myocardial ischemia. In addition to the many approaches aimed at increasing energy supply or decreasing energy demand, novel approaches include switching the heart to a more "oxygen-efficient" utilization of energy substrates (Lopaschuk et al., 2010). Adenosine receptor signaling has been extensively studied as an approach to treat myocardial ischemia (Eckle et al., 2008), with favorable alterations in cardiac energy metabolism contributing to the cardioprotective efficacy of adenosine receptor activation (Finegan et al., 1996).

A recent study (Eckle et al., 2012) provides important insights into the signaling pathways by which adenosine receptor 2b (Adora2b) activation mediates cardioprotection. The authors demonstrate a selective upregulation of Adora2b in clinical samples from patients with ischemic heart disease. To delineate the signaling pathways downstream of Adora2b activation and their potential role in cardioprotection the authors employed an ischemic preconditioning (IPC) protocol (adenosine receptor activation has been identified as a key component of IPC), where brief, sublethal periods of ischemia and reperfusion, prior to a

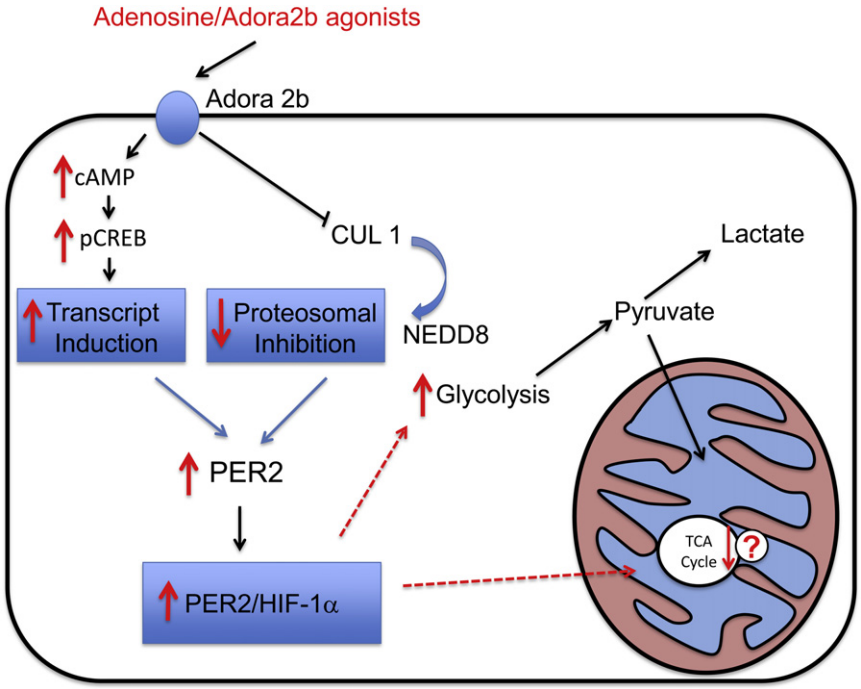

Figure 1. A Model for the Stimulation of Glycolysis in the Heart by Adora2b
Binding of adenosine to Adora2b increases cAMP levels and inhibits CUL 1 (via deneddylation). This results in an increase in phosphorylation of CREB, increased transcript induction of the circadian rhythm protein period 2 (Per2), and a stabilization of Per2 via proteasomal inhibition due to decreased ubiquitination of Per2. Increased levels of Per2 then stabilize HIF-1α, resulting in an induction of glycolytic enzymes and a stimulation of glycolysis. In parallel, a decrease in mitochondrial TCA cycle activity is observed. The increase in glycolysis may enable a more "oxygen-efficient" energy metabolism, affording cardioprotection to the heart during and following ischemia. Abbreviations: Adora2b, adenosine receptor 2b; cAMP, 3′-5′ cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; pCREB, phosphorylated cAMP response element-binding protein; CUL 1, cullin 1; HIF-1α, hypoxia-inducible factor-1α; NEDD8, ubiquitin-like polypeptide Nedd8; Per2, period 2; TCA, tricarboxylic acid.

prolonged, and otherwise lethal period of ischemia, decrease cardiac ischemia-reperfusion injury (Murry et al., 1986). RNA microarray and canonical pathway analysis identified the circadian rhythm protein, Per2, as exhibiting the highest differential readout (upregulation), between wild-type (WT) and *Adora2b*−/− mice subjected to cardiac IPC. The authors then demonstrate that Adora2b activation stabilizes Per2 by preventing its proteasomal degradation. Furthermore, they show that Per2, in turn, promotes the stabilization of hypoxia-inducible factor-1α (HIF-1α), leading to the induction of HIF-1α-target genes, including those encoding glycolytic enzymes. Importantly, the stabilization of HIF-1α and the upregulation of glycolysis seen with Adora2b stimulation and IPC were abolished in *per2*−/− mice, demonstrating that Per2 is required for the effects of IPC and Adora2b on glycolysis. Together these results show that this pathway enhances the glycolytic capacity of the ischemic heart, and the authors propose that this enhancement of glycolysis increases cardiac efficiency and the ability of the heart to adapt to ischemia. Combined, these data provide compelling evidence for a link between Adora2b activation, Per2 and HIF-1α stabilization, enhanced glycolysis, and cardioprotection (Figure 1).

A possible role for circadian rhythm in modulating ischemic injury was suggested by previous studies demonstrating that the extent of myocardial infarction is larger in the early-morning hours (Reiter et al., 2012). The involvement of a circadian rhythm protein (Per2) in modulating ischemic injury, as well as the observation that HIF-1α has a circadian pattern of expression, further implicates circadian-rhythm changes as a causative factor for the larger infarcts in patients suffering a myocardial infarction in the early morning (Eckle et al., 2012). However, the circadian oscillations of Per2 and HIF-1α proteins do not necessarily correlate with the timing of the increased infarct size observed in humans (Suárez-Barrientos et al., 2011). Interestingly, the authors did observe an increase in Per2 expression in patients with ischemic heart disease (Eckle et al., 2012), but did not test for potential circadian oscillations in Per2 expression. Whether the increase in Per2 expression in these patients is an adaptive response to ischemia or a nonspecific consequence also remains to be determined.

While increases in glycolysis have been linked to ischemic cardioprotection (Ashrafian et al., 2007), the role of glycolysis in adaptation to ischemia is not straightforward. During ischemia, the decrease in mitochondrial oxidative metabolism (due to oxygen limitation) results in an uncoupling of glycolysis from glucose oxidation, and the production of lactate, and H^+ ions from the hydrolysis of glycolytically derived ATP (Lopaschuk et al., 2010). As a result, while enhancing glycolysis can produce ATP without the need for oxygen, glycolysis can also be a major source of acidosis during and following ischemia. Not only can acidosis decrease cardiac efficiency (as ATP is redirected toward pathways that clear the H^+ and re-establish ionic homeostasis in the cardiac myocyte), it can also contribute to cell death, if severe. Under ideal conditions, therefore, glycolysis is coupled to glucose oxidation (i.e., the subsequent oxidation of the pyruvate derived from glycolysis) and neither lactate nor H^+ accumulate in the heart. This issue of glycolysis and glucose oxidation has interesting implications with regards to the downstream signaling of Adora2b and Per2. The data of Eckle et al. (2012) show that glycolytic enzyme induction and glycolysis during reperfusion following ischemia is impaired in *per2*−/− mice, yet the subsequent oxidation of glucose (measured using ^{13}C-glucose isotopomer analysis) is actually increased, an effect that should improve the coupling between glycolysis and glucose oxidation. Furthermore, stimulating glucose oxidation has been demonstrated to limit ischemia-reperfusion injury and decrease myocardial infarct size (Ussher et al., 2012), and may contribute to the cardioprotection in *per2*−/−mice observed by others (Virag et al., 2010). Eckle et al. (2012) also demonstrated

that increases in HIF-1α levels were associated with an increase in pyruvate dehydrogenase kinase 1 levels, which would be expected to inhibit glucose oxidation, since this kinase phosphorylates and inhibits the rate-limiting enzyme for glucose oxidation, pyruvate dehydrogenase. These results suggest that the Adora2b and Per2 signaling pathway, while increasing glycolysis, may actually impair glucose oxidation (Figure 1), as Per 2 deletion appears to enhance glucose oxidation. How the increase in glycolysis together with the probable decrease in glucose oxidation affects cardiac function during reperfusion following ischemia will require further clarification.

The effects of adenosine and adenosine receptor activation on cardiac glucose metabolism have been extensively studied. Activation of either adenosine receptor, Adora1 or Adora2, provides cardioprotection. Yet, Adora1, unlike Adora2, decreases glycolytic rates in the heart and improves coupling between glycolysis and glucose oxidation (Finegan et al., 1996). On the surface, these results appear to be at odds with the cardioprotection observed with Adora2b stimulation, which, as shown by Eckle et al. (2012), promotes glycolysis in the heart. As such, further studies are necessary to examine the relationship between adenosine receptor signaling, glucose metabolism, and cardioprotection. Nevertheless, the involvement of Per2 and HIF-1α, and the potential circadian rhythm of this pathway provide important insights into the control of glycolysis, and the potential role of these changes in adaptation to myocardial ischemia.

REFERENCES

Ashrafian, H., Frenneaux, M.P., and Opie, L.H. (2007). Circulation *116*, 434–448.

Eckle, T., Hartmann, K., Bonney, S., Reithel, S., Mittelbronn, M., Walker, L.A., Lowes, B.D., Han, J., Borchers, C.H., Buttrick, P.M., et al. (2012). Nat. Med. *18*, 774–782.

Eckle, T., Köhler, D., Lehmann, R., El Kasmi, K., and Eltzschig, H.K. (2008). Circulation *118*, 166–175.

Finegan, B.A., Lopaschuk, G.D., Gandhi, M., and Clanachan, A.S. (1996). Br. J. Pharmacol. *118*, 355–363.

Lopaschuk, G.D., Ussher, J.R., Folmes, C.D., Jaswal, J.S., and Stanley, W.C. (2010). Physiol. Rev. *90*, 207–258.

Murry, C.E., Jennings, R.B., and Reimer, K.A. (1986). Circulation *74*, 1124–1136.

Reiter, R., Swingen, C., Moore, L., Henry, T.D., and Traverse, J.H. (2012). Circ. Res. *110*, 105–110.

Suárez-Barrientos, A., López-Romero, P., Vivas, D., Castro-Ferreira, F., Núñez-Gil, I., Franco, E., Ruiz-Mateos, B., García-Rubira, J.C., Fernández-Ortiz, A., Macaya, C., and Ibanez, B. (2011). Heart *97*, 970–976.

Ussher, J.R., Wang, W., Gandhi, M., Keung, W., Samokhvalov, V., Oka, T., Wagg, C.S., Jaswal, J.S., Harris, R.A., Clanachan, A.S., et al. (2012). Cardiovasc. Res. *94*, 359–369.

Virag, J.A., Dries, J.L., Easton, P.R., Friesland, A.M., DeAntonio, J.H., Chintalgattu, V., Cozzi, E., Lehmann, B.D., Ding, J.M., and Lust, R.M. (2010). Am. J. Physiol. Heart Circ. Physiol. *298*, H1088–H1095.

Regulatory T Cells Expressing PPAR-γ Control Inflammation in Obesity

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A recent study (Cipolletta et al., 2012) shows that regulatory T (Treg) cells expressing the peroxisome-proliferator- activated receptor (PPAR-γ) are engaged in suppressing adipose tissue inflammation in obesity, suggesting that Treg cells may be a target for treatment and prevention of adipose tissue inflammation and insulin resistance.

In obesity, enlarged adipocytes accumulating in visceral adipose tissue (VAT) elicit infiltration of macrophages and other immune cells (Feuerer et al., 2009, Winer et al., 2009, Nishimura et al., 2009, Olefsky and Glass, 2010). These cells secrete proinflammatory cytokines and mediate chronic low-grade inflammation in VAT. The inflamed adipose tissue, in turn, may release cytokines, adipokines, fatty acids, and other substances that may affect other organs, such as liver and muscle, leading to systemic insulin resistance. A recent study from Cipolletta and colleagues reveals an important role for VAT-specific natural Treg cells in the suppression of obesity-associated inflammation in VAT and consequently in combatting insulin resistance (Cipolletta et al., 2012).

Naturally occurring Treg cells are a unique CD4+ T cell subpopulation specifically adapted to the suppression of aberrant or excessive immune responses that are harmful to the host (Sakaguchi et al., 2008). In physiological conditions, they constitute ~10% of peripheral CD4+ T cells and are characterized by the expression of the transcription factor Foxp3. The majority of Foxp3+ CD4+ Treg cells are produced by the thymus as a functionally mature and distinct T cell subpopulation, although naive conventional T cells can